THE LEAF ESSENTIAL OIL OF *JUNIPERUS ZANONII* AND COMPARISIONS WITH THE OILS OF *J. JALISCANA, J. MONTICOLA* AND *J. SALTILLENSIS*

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ABSTRACT

The first report on the composition of the leaf essential oil of *Juniperus zanonii* is presented and the oil compared to the oils of closely related species: *J. jaliscana, J. monticola* f. *monticola, J. m.* f. *compacta, J. m.* f. *orizabensis and J. saltillensis.* The leaf oil of *J. zanonii* is dominated by sabinene (30.9%), terpinen-4-ol (13.5%) and manoyl oxide (11.7%) with moderate amounts of γ-terpinene (5.6%), citronellal (4.3%), and abietatriene (3.4%). *Juniperus saltillensis* oil is dominated by camphor (44.5%) and α-pinene (12.1%) with moderate amounts of sabinene (7.7%), limonene (2.6%) and camphene hydrate (2.2%). The oils of the monticolan junipers (*J. m.* f. *monticola, J. m* f. *compacta, J. m.* f. *orizabensis*) are dominated by bornyl acetate (34.8, 44.2, 10.7%) and contain three compounds not found in other taxa in this study: δ-2-carene, linalyl acetate and methyl citronellate. However, these three forms have a surprising amount of variation among them. The oil of *J. jaliscana* is dominated by α-pinene (49.5%), limonene (15.1%) and β-phellandrene (10.0%). *Phytologia 92(2): 256-265 (August 2, 2010).*

KEY WORDS: *Juniperus zanonii, J. jaliscana, J. monticola* f. *monticola, J. m.* f. *compacta, J. m.* f. *orizabensis, J. saltillensis*, leaf essential oil composition, Cupressaceae, terpenes.

Recently, Adams et al. (2010) recognized the shrubby subalpine juniper from Cerro Potosi and adjacent peaks as a new species, *Juniperus zanonii* R. P. Adams, distinct from the *J. monticola* Mart. f. *compacta* Mart. from the trans-volcanic region of central Mexico. This was based in part on analyses of nrDNA and trnC-trnD sequences (Adams 2008) of the serrate leaf margined junipers of the western hemisphere that indicated the closest relative to the *J. zanonii* was *J. saltillensis* M. T. Hall (Fig. 1), not *J. monticola* Mart. In fact, *J. monticola* appears in a clade with *J. jaliscana* Mart. (Fig. 1).

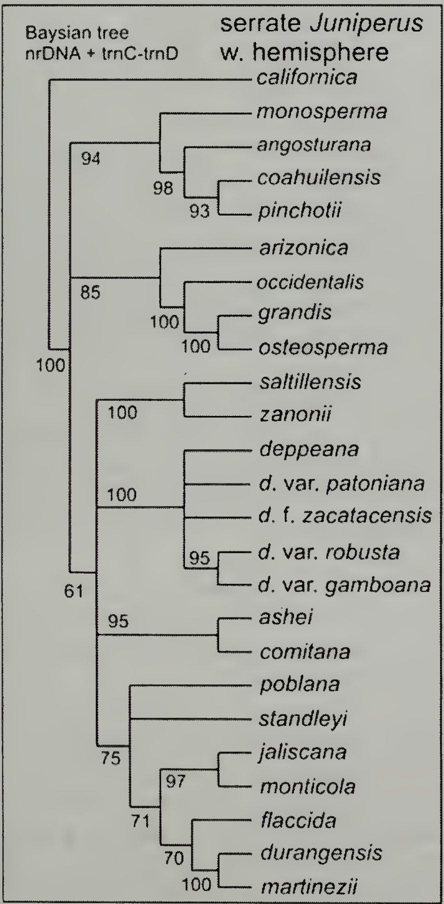

Figure 1. Bayesian tree for the serrate-leave *Juniperus* of the western hemisphere (modified from Adams, 2008) based on nrDNA + trnC-trnD. *J. zanonii* is in a well supported clade with *J. saltillensis*, whereas *J. monticola* (f. *monticola*) from El Chico National Park, Hidalgo, is in a clade with *J. jaliscana*.

A comparison of *J. zanonii* with *J. jaliscana, J. monticola f. monticola, J. monticola f. compacta and J. saltillensis* using 27 SNPs from nrDNA and petN-psbM (Adams et al., 2010) revealed (Fig. 2) that *J. zanonii* is not closely related to *J. monticola* f. *compacta* (from Pico Ixtaccihuatl) and that plants of *J. m.* f. *monticola* and f. *compacta* could not be resolved (Fig. 2) using these sequences.

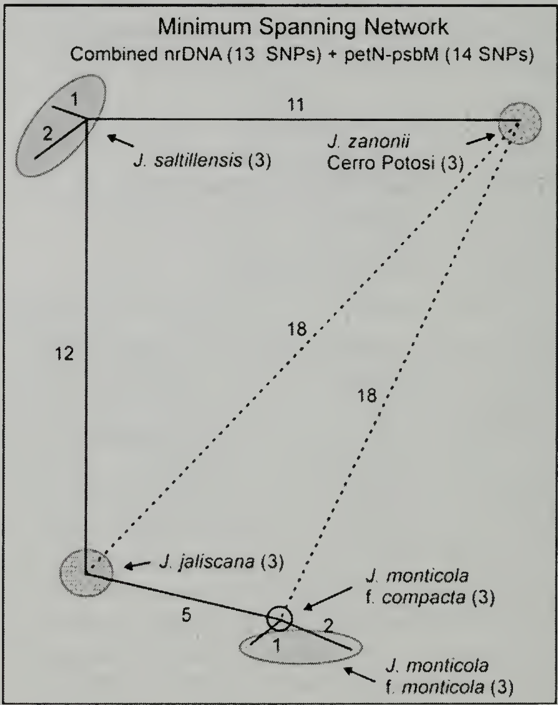

Figure 2. Minimum spanning network based on 27 SNPs from nrDNA and petN-psbM. Adapted from Adams et al. (2010).

The compositions of the leaf essential oils of the related taxa have been reported: *J. jaliscana* (Adams, Zanoni and Hogge, 1985); *J. monticola* f. *monticola, J. m.* f. *compacta, J. m.* f. *orizabensis* (Adams et al. 1980a) and *J. saltillensis* (Adams et al. 1980b). A summary of the compositions of the leaf essential oils of the serrate junipers of the western hemisphere has been presented by Adams (2000). It is worth noting that many unknown compounds reported in these early papers are now indentified in this updated examination of the aforementioned oils.

The purpose of the present study is to present the first analysis of the leaf essential oil of *J. zanonii* and compare its composition with that of closely related species: *J. jaliscana, J. monticola* f. *monticola, J. m.* f. *compacta, J. m.* f. *orizabensis and J. saltillensis.*

MATERIALS AND METHODS

Specimens collected: *J. jaliscana*, *Adams 6846-6848*, 12/12/1991, 940 m, 19 km E of Mex. 200 on the road to Cuale, Jalisco, Mexico; *J. monticola* f. *compacta*: *T. A. Zanoni 2601-2618,* Pico Ixtaccihuatl, Mexico; *J. monticola* f. *monticola*, *Adams 6874-6878*, 12/20/1991, 2750 m, El Chico National Park, Hidalgo, Mexico; *J. monticola* f. *orizabensis*: *T. A. Zanoni 2627-2636*, Pico de Orizaba, Vera Cruz, Mexico; *J. saltillensis*, *Adams 6886-6890*, 12/21/1991, 2090 m, on Mex. 60, 14 km E. of San Roberto Junction, Nuevo Leon, Mexico; *J. zanonii*, *Adams 6898-6902*, 12/21/1991, 3490 m, Cerro Potosi, Nuevo Leon, Mexico. Voucher specimens are deposited at BAYLU.

Isolation of Oils - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

Chemical Analyses - The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see 5 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

RESULTS AND DISCUSSION

The compositions of the leaf oils are shown in Table 1. The leaf oil of *J. zanonii* is dominated by sabinene (30.9%), terpinen-4-ol (13.5%) and manoyl oxide (11.7%) with moderate amounts of γ-terpinene (5.6%), citronellal (4.3%), and abietatriene (3.4%).

Juniperus saltillensis leaf essential oil is dominated by camphor (44.5%) and α-pinene (12.1%) with moderate amounts of sabinene (7.7%), limonene (2.6%) and camphene hydrate (2.2%).

The oil of *J. jaliscana* is dominated (Table 1) by α-pinene (49.5%), limonene (15.1%) and β-phellandrene (10.0%) with moderate amounts of myrcene (4.5%) and β-pinene (2.3%).

The oils of the monticolan junipers (*J. m.* f. *monticola, J. m* f. *compacta, J. m.* f. *orizabensis*) are dominated by bornyl acetate (34.8, 44.2, 10.7%) and contain three compounds not found in other taxa in this study: δ-2-carene, linalyl acetate and methyl citronellate. The oil of *J. m. f. monticola* also contains moderate amounts of α-pinene (15.1%), limonene (6.7%), β-phellandrene (6.8%) and elemol (5.4%), whereas f. *compacta* contains moderate amounts of sabinene (17.8%), β-phellandrene (6.3%), terpinen-4-ol (9.8%), elemol (5.7%) and manoyl oxide (6.3%) and f. *orizabensis* has α-pinene (6.5%), limonene (7.9%), β-phellandrene (7.8%) and camphor (4.4%). Despite the seemingly small amount of variation seen in the DNA sequences for nrDNA and petN-psbM (Fig. 2), these three forms have a surprising amount of variation in their leaf essential ols.

It is interesting that the oil of *J. m.* f. *compacta* shares some similarity to *J. zanonii*. Note the similar amounts of sabinene, terpinen-4-ol, manoyl oxide and abietatriene (Table 1). However, *J. m* f. *compacta* and *J. zanonii* differ considerably in the concentrations of borneol, bornyl acetate, β-phellandrene, citronellal and many trace components (Table 1).

ACKNOWLEDGEMENTS

This research was supported in part with funds from Baylor University. Thanks to Tonya Yanke for lab assistance.

LITERATURE CITED

Adams, R. P. 1991. Cedarwood oil - Analysis and properties. pp. 159-173. in: Modern Methods of Plant Analysis, New Series: Oil and Waxes. H.-F. Linskens and J. F. Jackson, eds. Springler- Verlag, Berlin.

Adams, R. P. 2000. The serrate leaf margined *Juniperus* (Section Sabina) of the western hemisphere: Systematics and evolution based on leaf essential oils and Random Amplified Polymorphic DNAs (RAPDs). Biochem. Syst. Ecol. 28: 975-989.

Adams, R. P. 2007. Identification of essential oil components by gas chromatography/ mass spectrometry. 2nd ed. Allured Publ., Carol Stream, IL.

Adams, R. P. 2008. The junipers of the world: The genus *Juniperus*. 2nd ed. Trafford Publ., Victoria, BC.

Adams, R. P., A. E. Schwarzbach and J. A. Morris. 2010. *Juniperus zanonii*, a new species from Cerro Potosi, Mexico. Phytologia 92: 105-117.

Adams, R. P., E. von Rudloff, L. R. Hogge, and T. A. Zanoni. 1980a. The volatile terpenoids of *Juniperus monticola f. monticola, f. compacta,* and f. *orizabensis*. J. Natl. Products 43:417-419.

Adams, R. P., E. von Rudloff, T. A. Zanoni and L. R. Hogge. 1980b. The terpenoids of an ancestral/advanced species pair of *Juniperus*. Biochem. Syst. Ecol. 8:35-37.

Adams, R. P., T. A. Zanoni and L. Hogge. 1985. The volatile leaf oils of two rare junipers from western Mexico: *Juniperus durangensis* and *J. jaliscana.* J. Nat. Prod. 48:673-674.

Table 1. Comparison of leaf essential oils of *J. jaliscana* (Jal), *J. monticola* f. *monticola* (MM), *J. m.* f. *compacta* (MC), *J. m.* f. *orizabensis* (MO), *J. saltillensis* (Salt) and *J. zanonii* (Zan). Compounds in bold appear to separate the taxa. t = trace, < 0.1%, RI = retention index on DB-5. Unidentified compounds have the four strongest ions listed.

RI	Component	Salt	Zan	MC	MO	MM	Jal
846	(E)-2-hexenal	-	-	-	0.3	-	0.1
846	(E)-3-hexenol	-	-	-	-	-	0.6
849	ethyl isovalerate	-	0.2	-	-	0.1	-
921	tricyclene	0.4	-	0.2	0.9	1.0	0.1
924	α-thujene	0.3	1.8	0.4	-	-	t
932	**α-pinene**	**12.1**	**3.0**	**4.5**	**6.5**	**15.1**	**49.5**
945	α-fenchene	-	-	-	-	-	t
946	camphene	0.8	t	0.3	1.8	1.4	0.5
961	verbenene	1.8	0.2	t	-	1.8	0.1
969	**sabinene**	**7.7**	**30.9**	**17.8**	**t**	**t**	**0.1**
974	β-pinene	1.3	0.6	0.5	0.2	0.9	2.3
988	myrcene	1.9	1.6	1.5	2.4	3.0	4.5
1001	**δ-2-carene**	**-**	**-**	**0.7**	**2.3**	**0.1**	**-**
1002	α-phellandrene	t	0.3	t	0.4	0.1	t
1008	δ-3-carene	0.4	-	0.2	0.2	t	t
1014	α-terpinene	0.5	0.3	1.2	0.2	0.1	t
1020	p-cymene	0.1	0.2	0.8	0.7	0.2	0.1
1024	**limonene**	**2.6**	**1.0**	**-**	**7.9**	**6.7**	**15.1**
1025	**β-phellandrene**	**1.3**	**1.1**	**6.3**	**7.8**	**6.8**	**10.0**
1026	1,8-cineole	0.1	-	-	-	-	-
1044	(E)-β-ocimene	0.1	-	-	0.1	0.2	0.2
1054	**γ-terpinene**	**0.8**	**5.6**	**2.4**	**0.4**	**0.6**	**0.4**
1063	unknown, <u>88</u>,101,43,158	0.2	-	-	0.7	1.0	-
1065	**cis-sabinene hydrate**	**0.3**	**0.9**	**1.0**	**-**	**t**	**-**
1067	cis-linalool oxide (furan-oid)	-	-	0.1	t	-	-
1086	terpinolene	0.7	1.7	-	0.7	0.8	1.1
1092	C10-OH, <u>96</u>,109,137,152	2.8	-	-	-	0.6	-
1095	linalool	0.6			1.4	1.1	0.2
1098	trans-sabinene hydrate	-	1.0	1.3	-	-	-
1100	n-nonanal	-	0.1	-	t	-	t
1102	isopentyl-isovalerate	-	0.1	0.1	-	-	-
1112	3-methyl-3-buten-methyl-butanoate	-	0.2	0.2	-	0.2	t
1114	endo-fenchol	-	-	-	-	-	t
1118	**cis-p-menth-2-en-1-ol**	**0.2**	**0.8**	**0.8**	**-**	**0.1**	**t**

RI	Component	Salt	Zan	MC	MO	MM	Jal
1122	α-campholenal	-	-	-	t	-	0.1
1135	trans-pinocarveol	-	-	-	-	-	0.1
1136	trans-p-menth-2-en-1-ol	-	0.5	0.5	0.9	0.1	-
1141	**camphor**	**44.5**	**0.1**	**1.6**	**4.4**	**1.0**	**0.1**
1144	neo-isopulegol	-	0.3	-	-	0.4	-
1145	camphene hydrate	2.3	t	0.2	1.4	1.0	0.1
1148	**citronellal**	**1.0**	**4.3**	**0.4**	**-**	**-**	**-**
1155	iso-pulegol	-	0.2	-	-	-	-
1165	**borneol**	**0.8**	**-**	**3.2**	**2.0**	**1.8**	**0.2**
1167	umbellulone	-	-	-	0.4	-	-
1172	cis-pinocamphone	0.1	-	-	-		t
1174	**terpinen-4-ol**	**1.9**	**13.5**	**9.8**	**0.7**	**0.6**	**0.1**
1179	p-cymen-8-ol	t	-	0.1	0.1	t	0.1
1186	α-terpineol	0.2	0.5	0.5	0.3	0.2	0.5
1193	4Z-decanal	0.2	-	-	-	-	-
1195	cis-piperitol	-	0.2	0.1	0.4	t	-
1195	myrtenol	-	-	-	-	-	t
1195	methyl chavicol	-	0.2	-	-	-	-
1207	trans-piperitol	-	0.4	0.5	0.5	0.1	-
1215	trans-carveol	-	-	-	-	-	t
1218	endo-fenchyl acetate	-	-	-	0.1	0.1	0.1
1223	**citronellol**	**1.5**	**1.4**	**0.3**	**-**	**t**	**-**
1232	thymol, methyl ether	-	-	0.1	t	-	0.1
1235	trans-chrysanthenyl acetate	0.5	-	-	-	1.1	-
1239	carvone	-	-	-	t	-	-
1241	carvacrol, methyl ether		-	-	-	-	t
1247	C10-OH, 41,109,119,152	0.6	-	-	-	-	-
1249	piperitone	-	-	0.2	0.4	-	-
1254	**linalyl acetate**	**-**	**-**	**t**	**0.2**	**0.1**	**-**
1255	4Z-decenol	0.2	-	-	-	-	-
1257	**methyl citronellate**	**-**	**-**	**0.2**	**t**	**0.1**	**-**
1274	pregeijerene B	0.5	1.0	0.9	0.3	0.9	-
1275	isopulegol acetate	-	-	-	-	0.1	-
1287	**bornyl acetate**	**0.9**	**0.1**	**10.7**	**44.2**	**34.8**	**1.1**
1289	thymol	0.1	-	0.3	-	-	-
1298	carvacrol	-	-	-	0.1	0.2	-
1312	citronellic acid	0.1	0.2	0.2	0.1	0.1	-
1346	**α-terpinyl acetate**	**-**	**-**	**0.2**	**0.6**	**0.1**	**-**
1403	methyl eugenol	t	0.3	-	-	-	-
1448	cis-muurola-3,5-diene	-	-	0.3	-	-	0.3
1417	(E)-caryophyllene	t	-	-	0.4	t	0.2

RI	Component	Salt	Zan	MC	MO	MM	Jal
1451	trans-muurola-3,5-diene	t	-	-	-	-	t
1452	α-humulene	-	-	-	-	-	0.3
1461	cis-cadina-1(6),4-diene	-	-	-	-	-	0.3
1465	cis-muurola-4(14),5-diene	-	-	-	-	-	0.5
1475	trans-cadina-1(6),4-diene	-	-	0.2	-	-	t
1480	germacrene D	-	-	-	-	-	1.1
1493	trans-muurola-4(14),5-diene	t	-	0.7	-	-	-
1493	epi-cubebol	-	-	0.4	-	-	-
1495	epi-cubebene	-	-	-	-	-	t
1500	α-muurolene	-	-	-	-	-	0.1
1501	epi-zonarene	-	-	-	-	-	0.1
1513	γ-cadinene	-	-	-	-	-	0.6
1513	cubebol	-	-	0.6	-	-	-
1521	trans-calamenene	-	-	-	-	-	0.1
1522	δ-cadinene	-	-	0.3	-	-	0.9
1528	zonarene	-	-	0.2	-	-	-
1531	cis-calamenene	-	-	t	-	-	-
1537	α-cadinene	-	-	-	-	-	0.1
1544	α-calacorene	-	-	-	-	-	0.1
1548	**elemol**	**1.4**	**2.5**	**5.7**	**2.4**	**5.4**	-
1559	germacrene B	-	-	-	0.1	0.2	-
1574	germacrene-D-4-ol	-	-	-	-	-	0.1
1582	caryophyllene oxide	-	-	-	0.1	-	t
1600	cedrol	-	-	t	0.2	-	-
1608	humulene epoxide II	-	-	-	-	-	t
1608	$C_{15}OH$, 43,109,119,220	0.1	-	-	0.5	0.6	-
1627	1-epi-cubenol	0.1	-	0.7	-	-	-
1630	γ-eudesmol	0.2	0.4	1.3	0.5	1.0	-
1638	epi-α-cadinol	-	-	-	-	-	1.1
1638	epi-α-muurolol	-	-	-	-	-	1.0
1644	α-muurolol	-	-	-	-	-	0.2
1649	β -eudesmol	0.3	0.4	2.6	0.5	0.9	-
1652	α-eudesmol	0.3	0.5	1.9	0.5	1.2	-
1652	α -cadinol	-	-	-	-	-	1.5
1670	bulnesol	0.1	0.1	0.4	0.3	0.5	0.1
1688	shyobunol	-	-	0.2	-	-	-
1746	8-α-11-elemodiol	0.3	0.2	0.2	-	0.2	-
1792	8-α-acetoxyelemol	0.3	0.7	2.0	0.5	1.2	-
1958	iso-pimara-8(14),15-diene	-	0.6	t	-	-	t
1988	**manoyl oxide**	**1.5**	**11.7**	**6.3**	**0.2**	**1.0**	**0.2**

RI	Component	Salt	Zan	MC	MO	MM	Jal
1987	iso-pimara-7,15-diene	-	-	-	0.2	0.5	-
2009	epi-13-manoyl oxide	-	0.1	t	-	-	-
2055	**abietatriene**	**0.3**	**3.4**	**3.1**	**0.3**	**1.1**	**0.3**
2087	abietadiene	-	0.3	t	t	0.5	0.1
2105	iso-abienol	0.3	-	1.4	-	0.1	-
2256	methyl sandaracopimarate	1.5	-	-	-	-	-
2282	sempervirol	0.4	0.1	0.1	-	-	-
2298	4-epi-abietal	-	-	0.1	-	t	t
2314	trans-totarol	0.2	t	0.6	0.1	0.7	0.6
2331	trans-ferruginol	0.1	t	0.1	-	t	t
	Number of cpds.	60	52	69	59	63	71